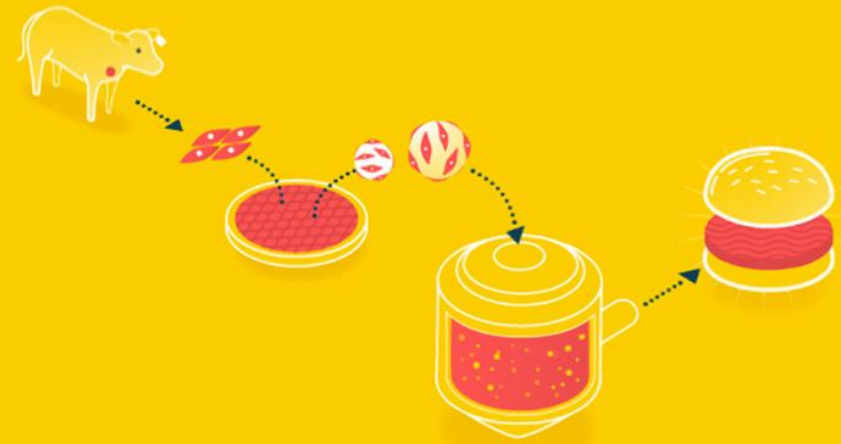


Tufts BME174 – Meat Lab

Week 5: Verification of Myogenicity
and Adipogenicity





Week 5

Part 1

- Muscle & fat differentiation assay background
- Lab report expectations

Part 2

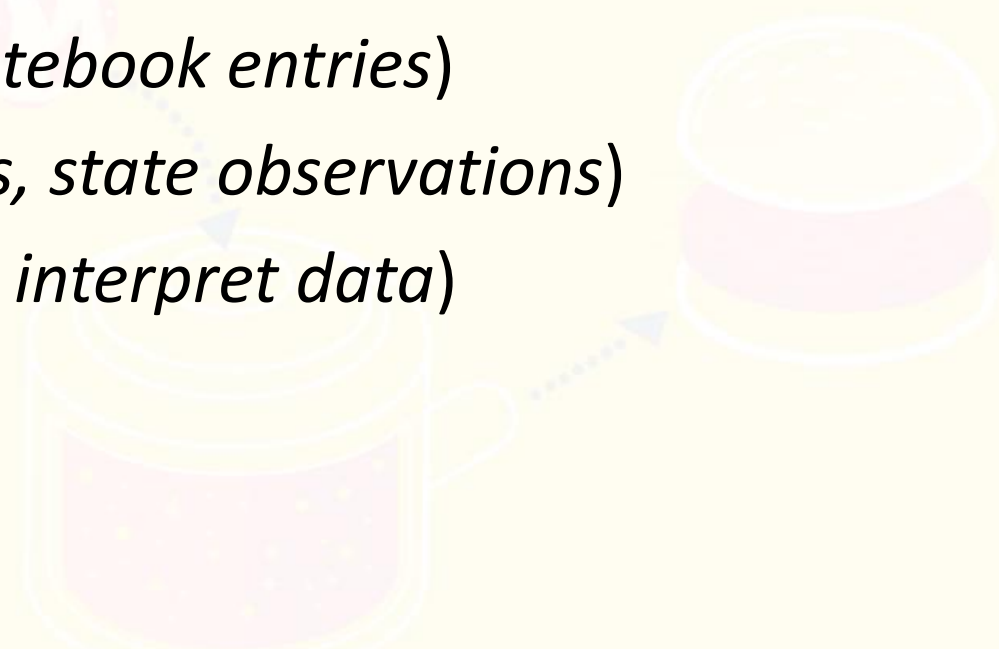
- LADD stain for muscle
- Oil Red O for fat

Lab Report Expectations

1. Introduction (*include references*)
2. Methods (*can reference lab notebook entries*)
3. Results (*include figure captions, state observations*)
4. Discussion (*include references, interpret data*)
5. References (*any format*)

Length: maximum 2,500 words

NEW *due date: March 6th (extended 1 week)*



What happened to your cultures since last week?

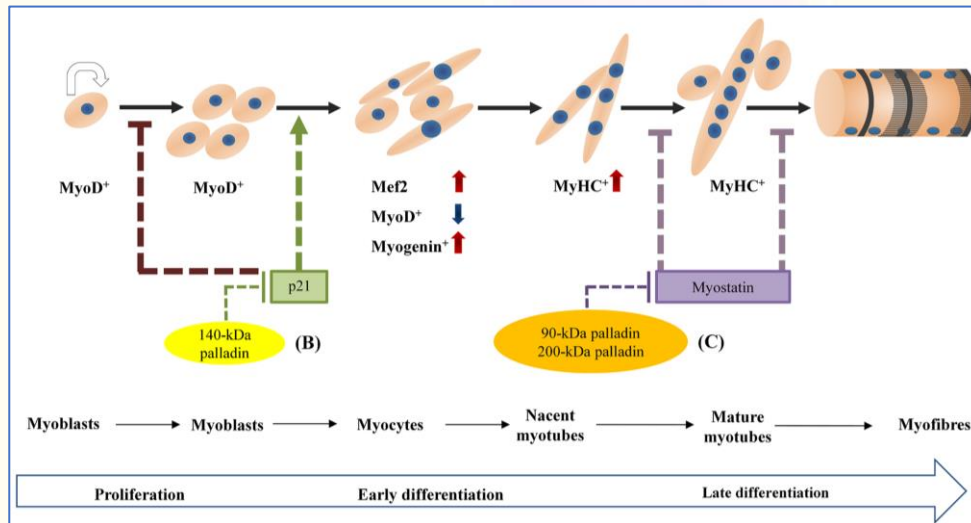
Muscle

Fat

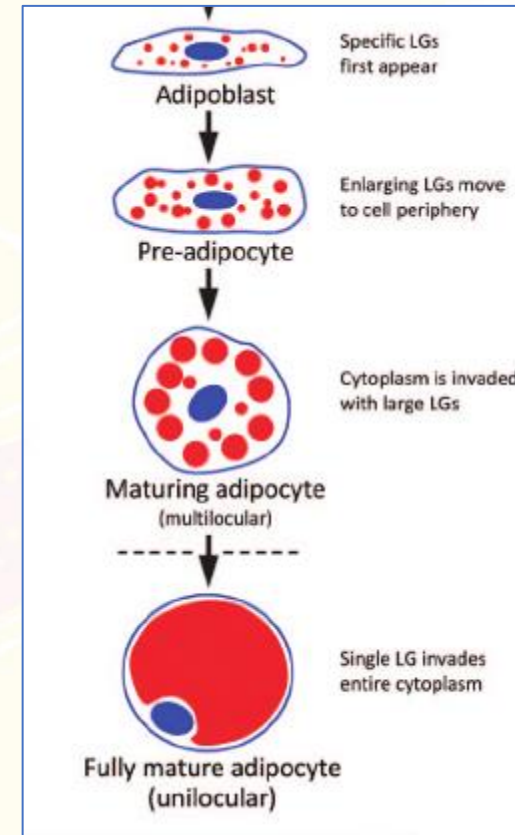


What happened to your cultures since last week?

Muscle



Fat

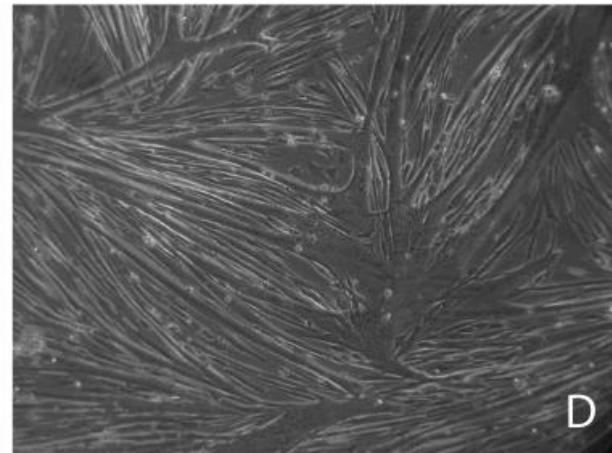
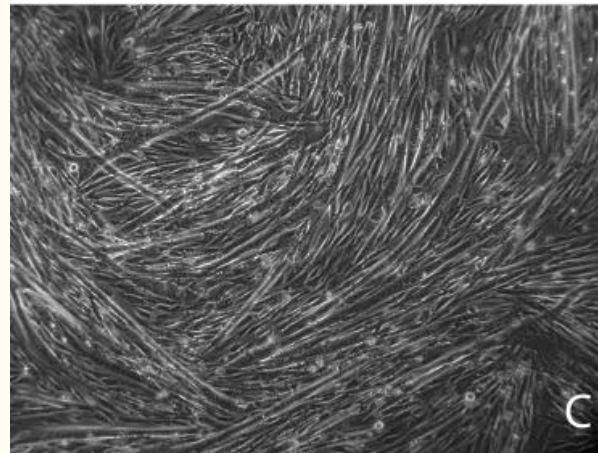
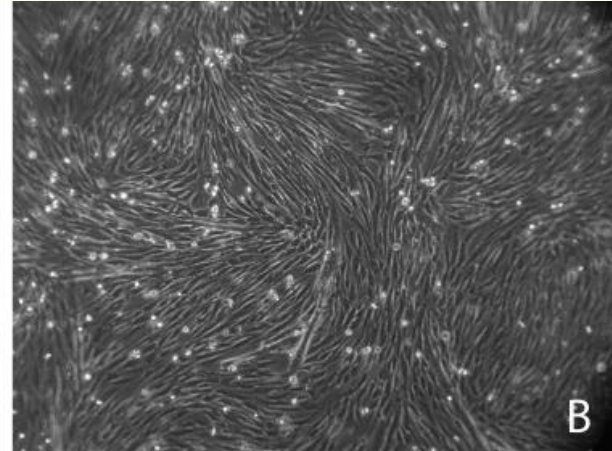
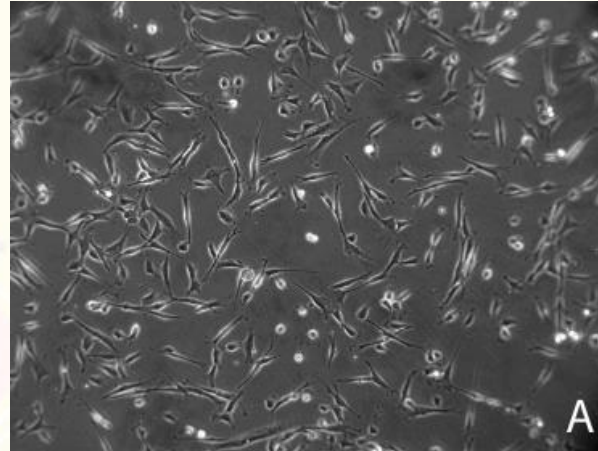


Methods to quantify muscle?



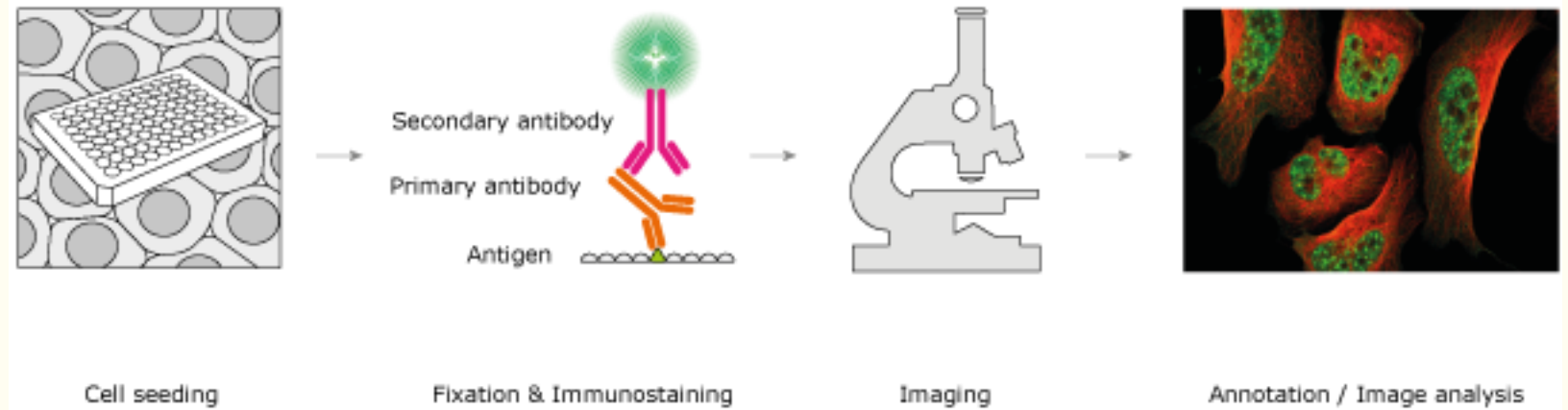
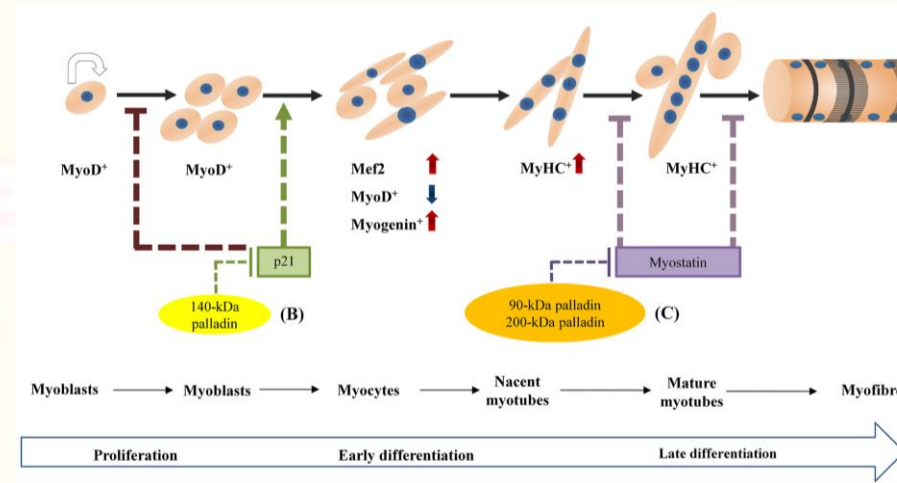
Methods to quantify muscle

- Morphology
- Immunostaining
- Western blot
- qPCR



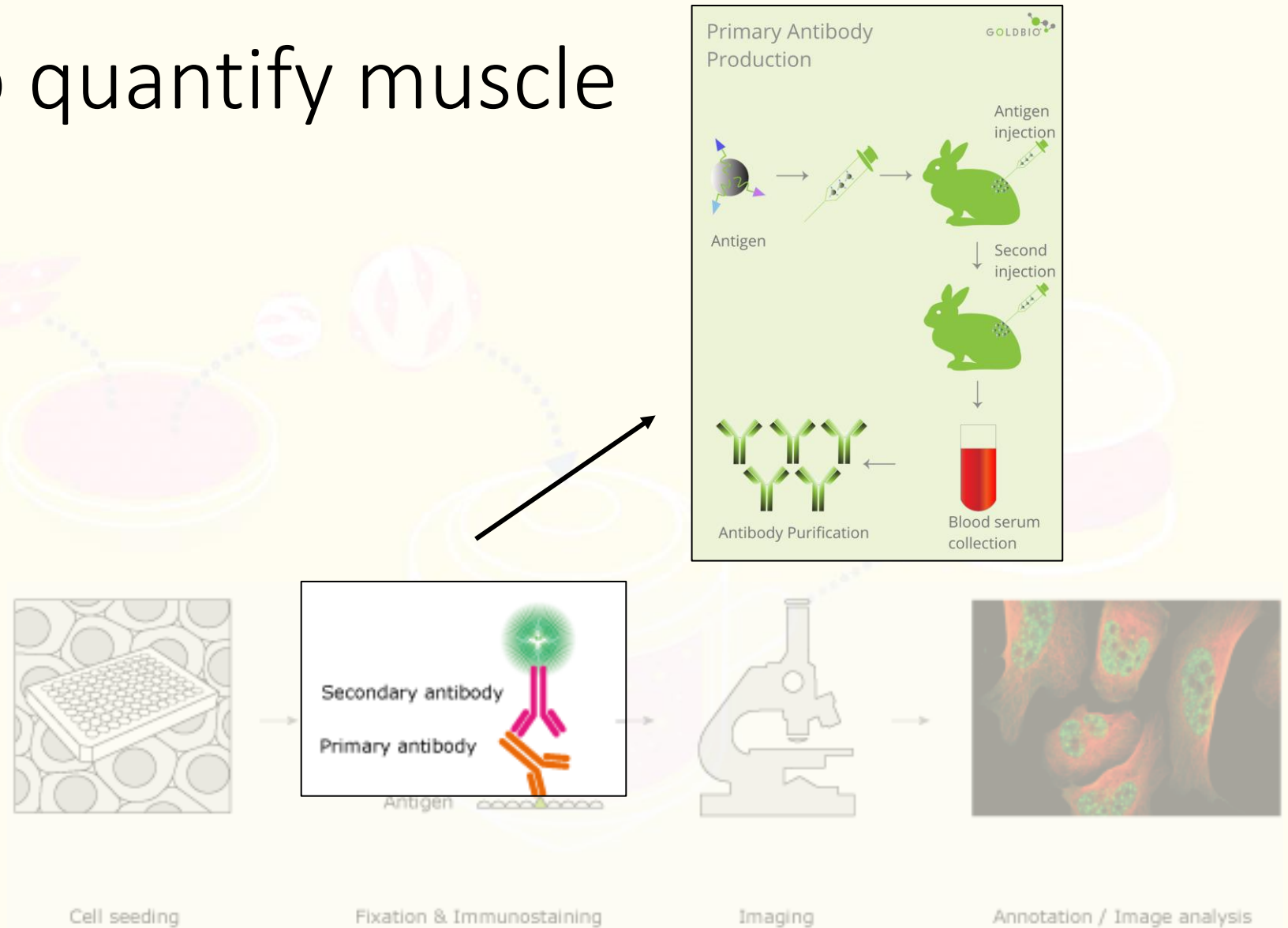
Methods to quantify muscle

- Morphology
- Immunostaining
- Western blot
- qPCR



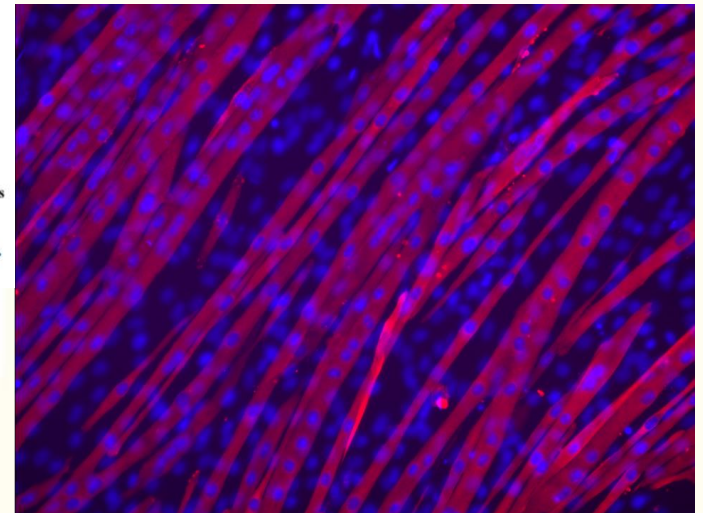
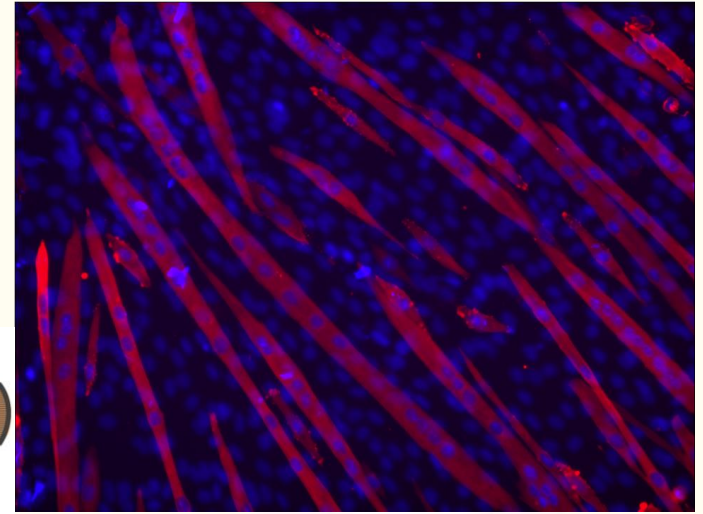
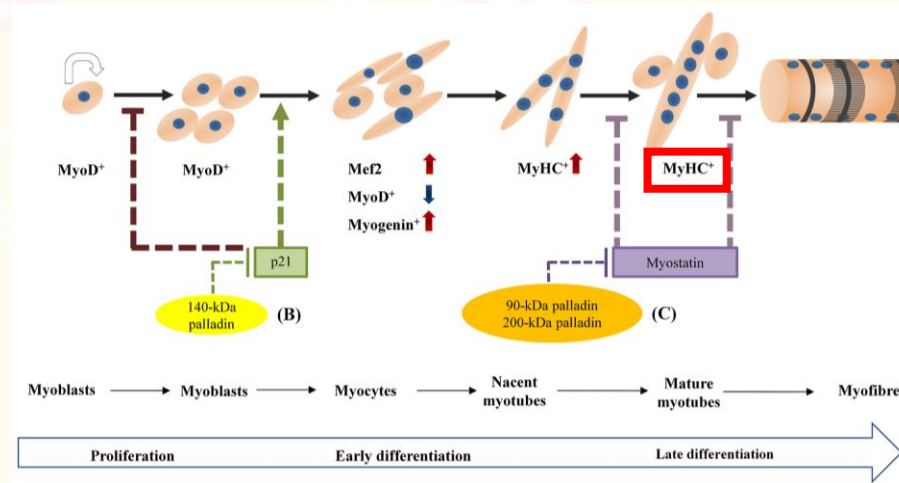
Methods to quantify muscle

- Morphology
- Immunostaining
- Western blot
- qPCR



Methods to quantify muscle

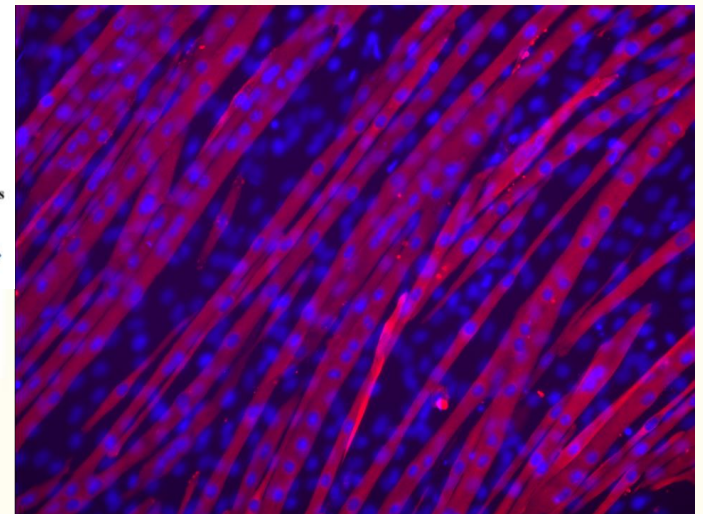
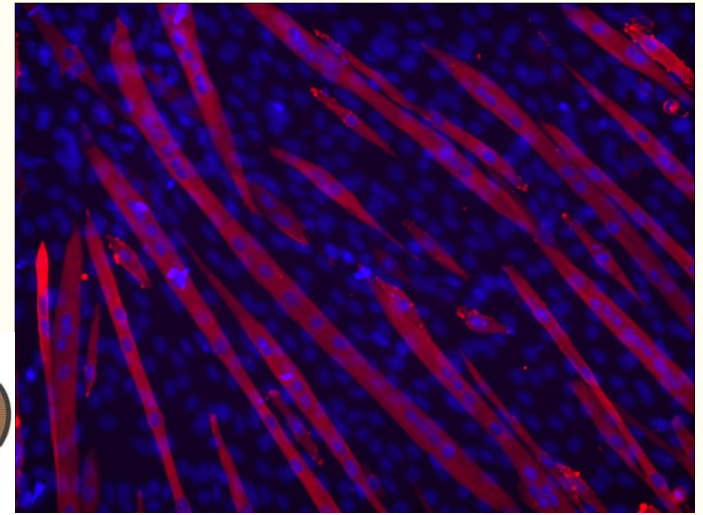
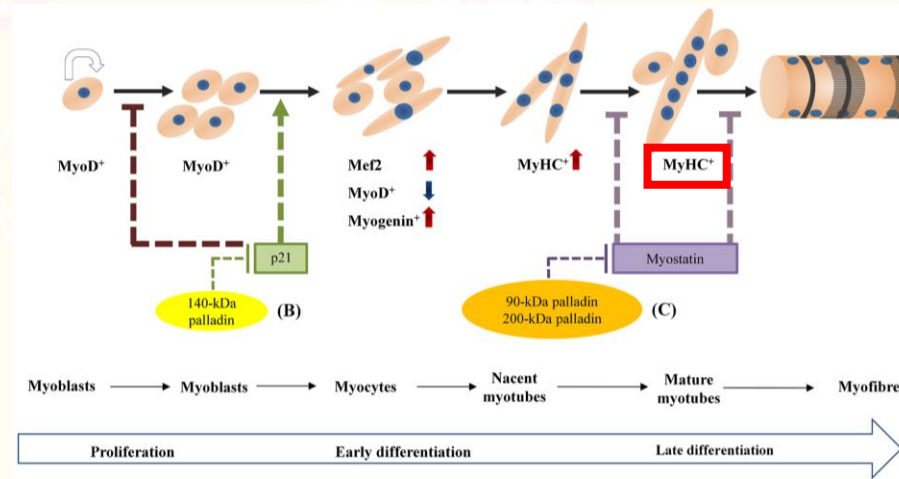
- Morphology
- Immunostaining
- Western blot
- qPCR



Myosin heavy chain
DAPI

Methods to quantify muscle

- Morphology
- Immunostaining
- Western blot
- qPCR

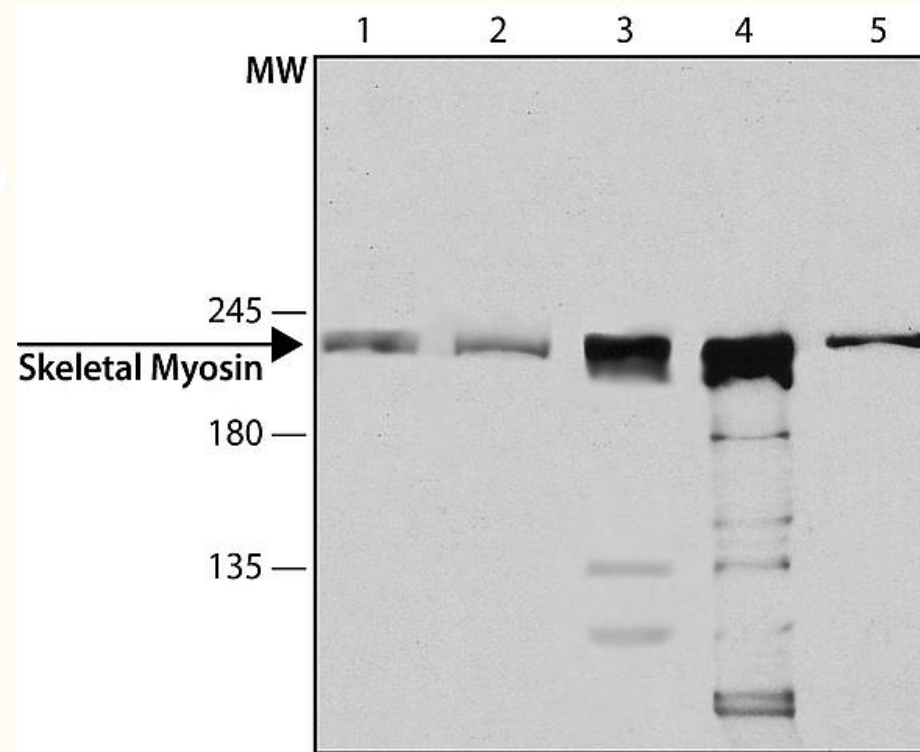


Myosin heavy chain
DAPI

Fusion index = total nuclei/nuclei within
MHC+ myotubes

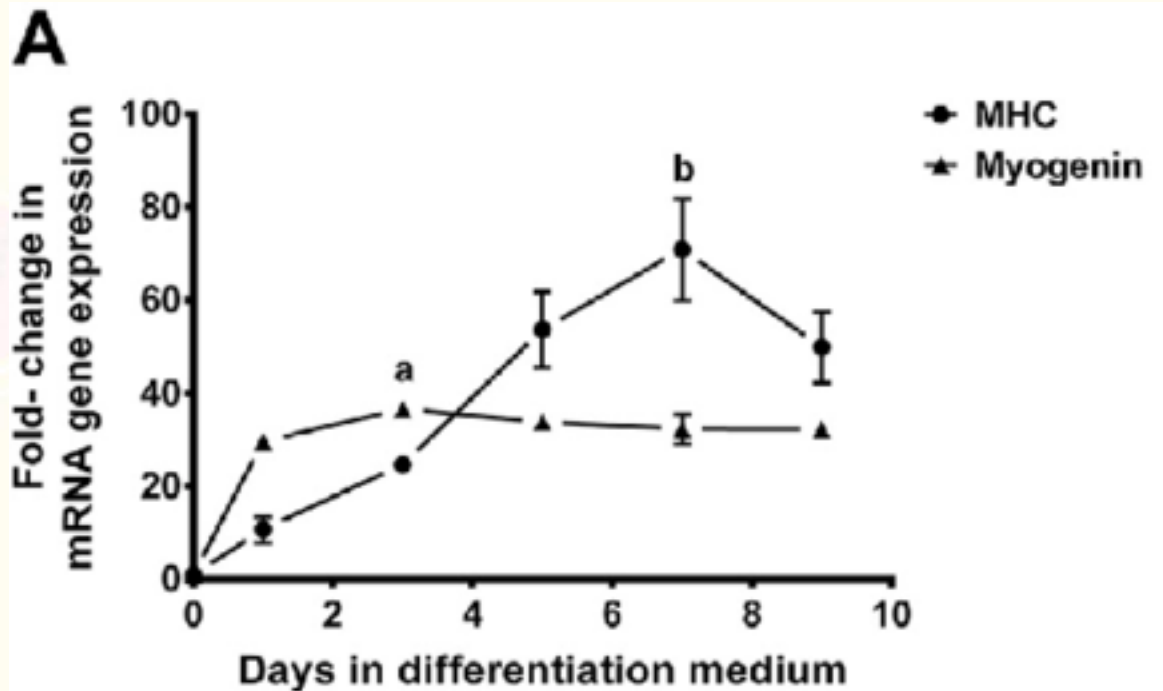
Methods to quantify muscle

- Morphology
- Immunostaining
- **Western blot**
- qPCR



Methods to quantify muscle

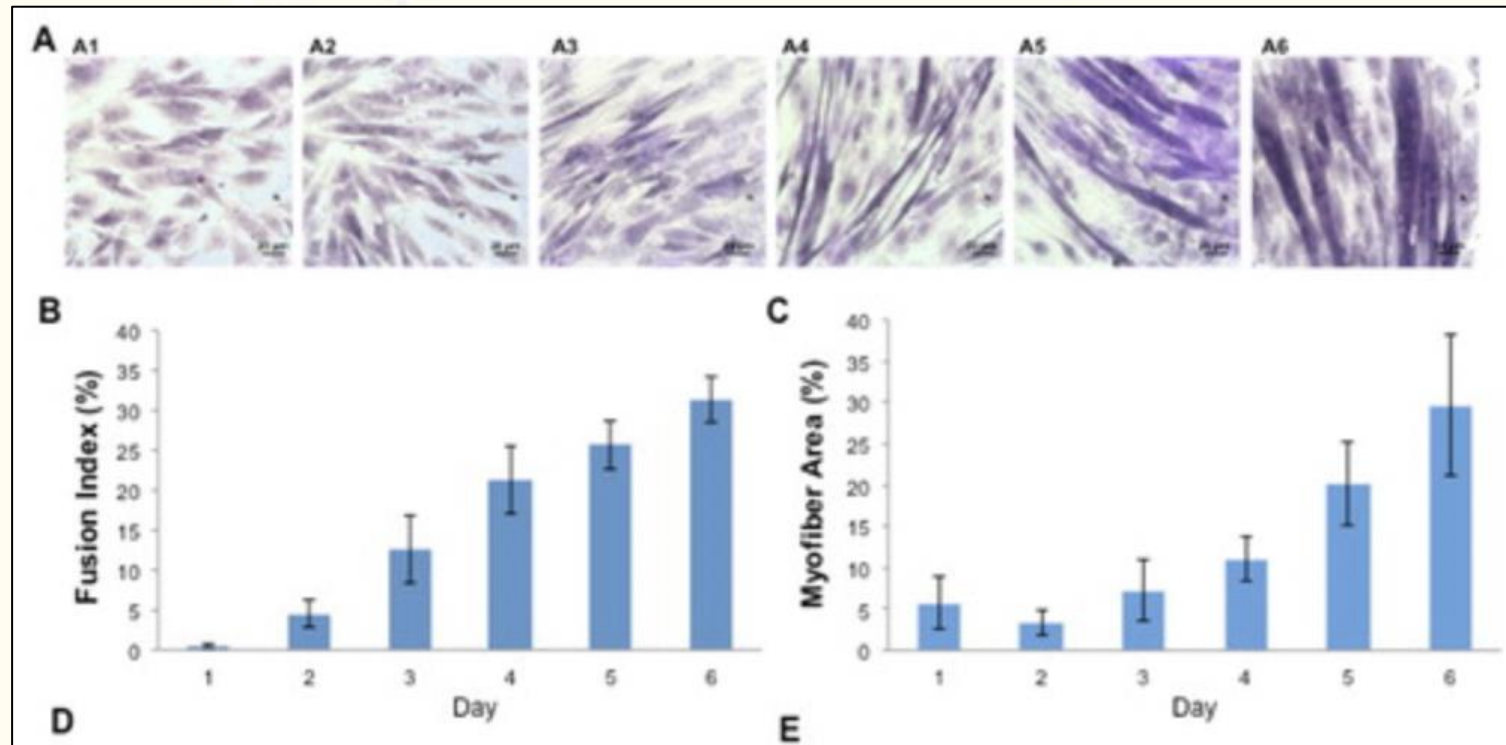
- Morphology
- Immunostaining
- Western blot
- qPCR



LADD stain: an alternative to immunostaining

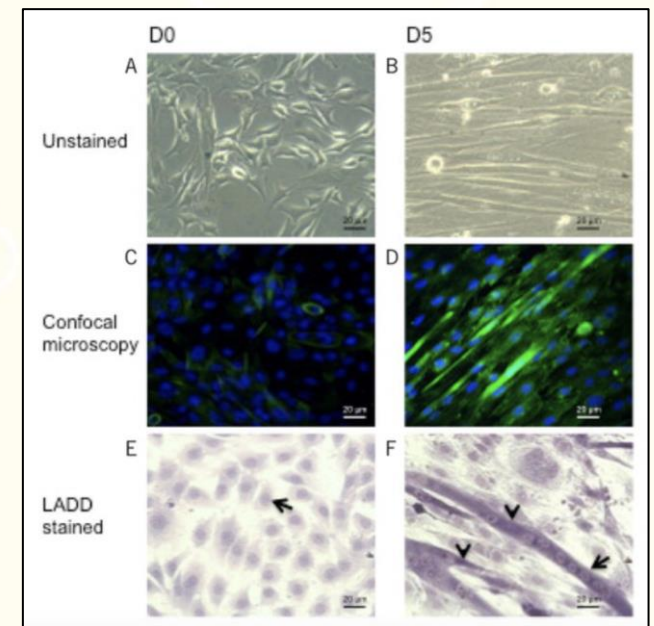


LADD stain: an alternative to immunostaining



Components:

1. Fuchsin
2. Toluidine blue



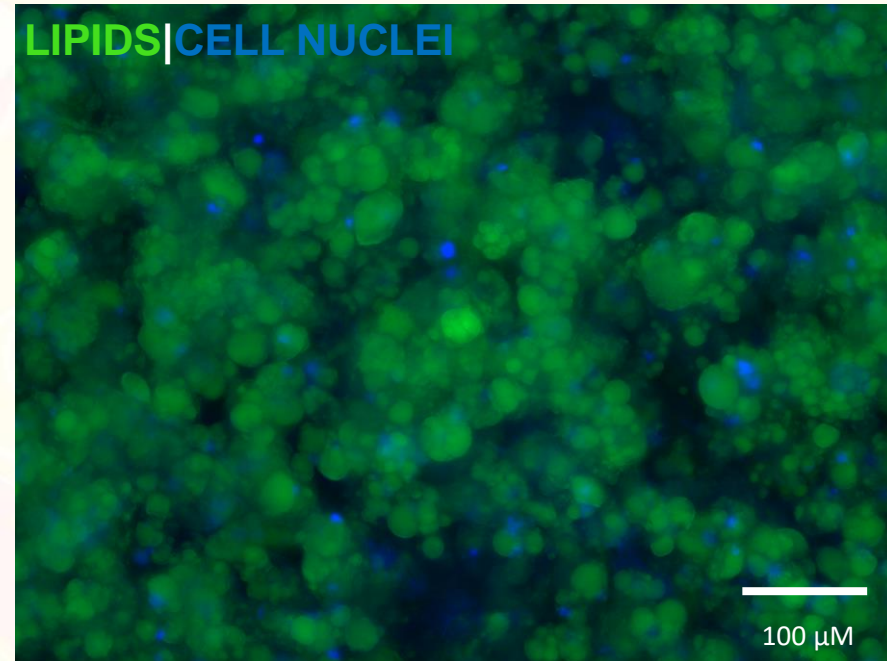
Methods to analyze fat

- BODIPY
- Immunostaining
- Lipidomics
- Oil Red O



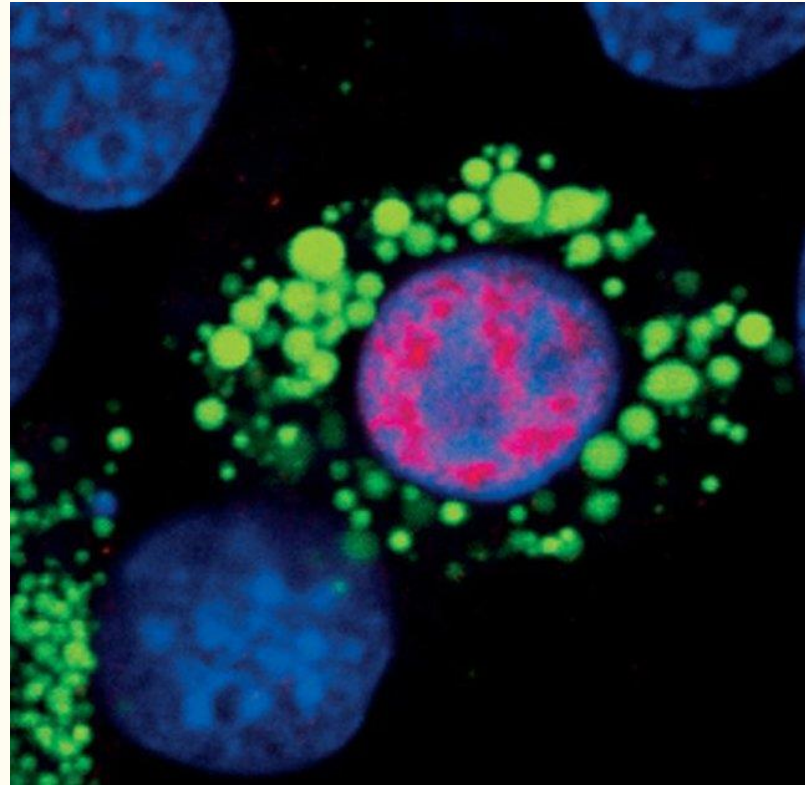
Methods to analyze fat

- BODIPY
- Immunostaining
- Lipidomics
- Oil Red O



Methods to analyze fat

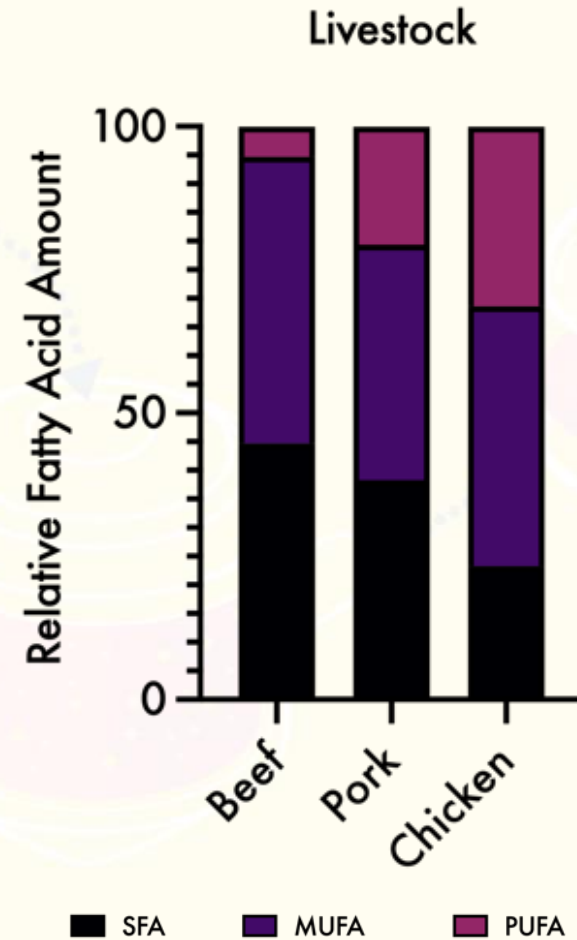
- BODIPY
- Immunostaining
- Lipidomics
- Oil Red O



BODIPY
PPAR gamma
Cell Nuclei

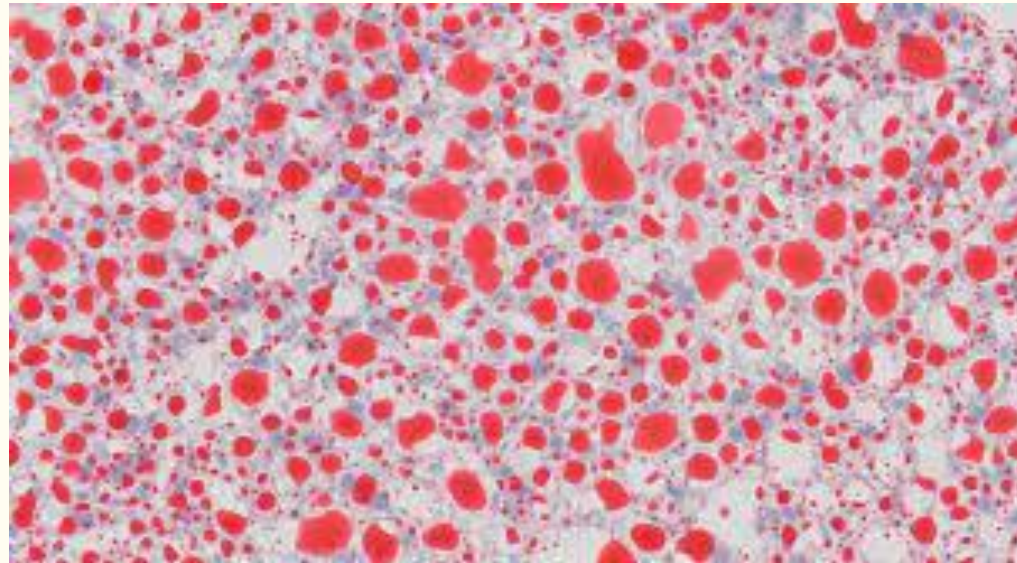
Methods to analyze fat

- BODIPY
- Immunostaining
- Lipidomics
- Oil Red O



Methods to analyze fat

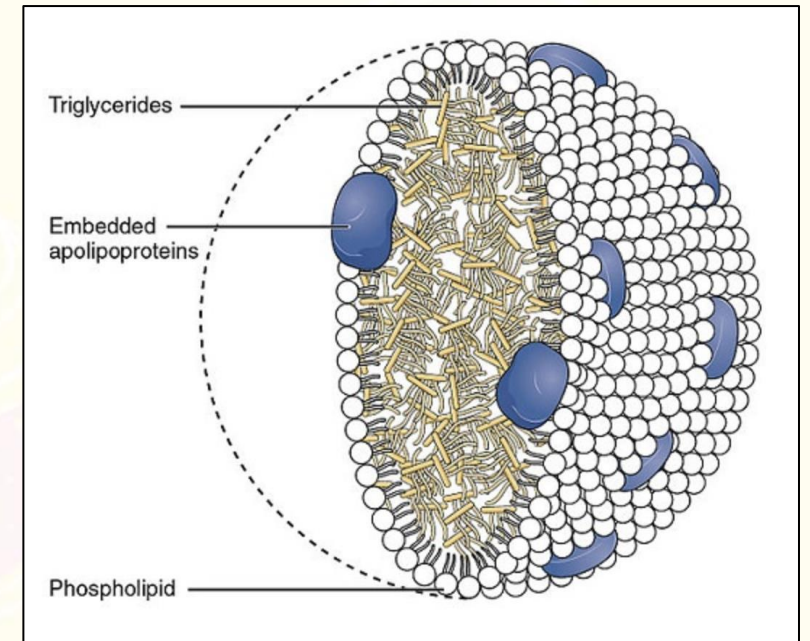
- BODIPY
- Immunostaining
- Lipidomics
- Oil Red O



Oil Red O

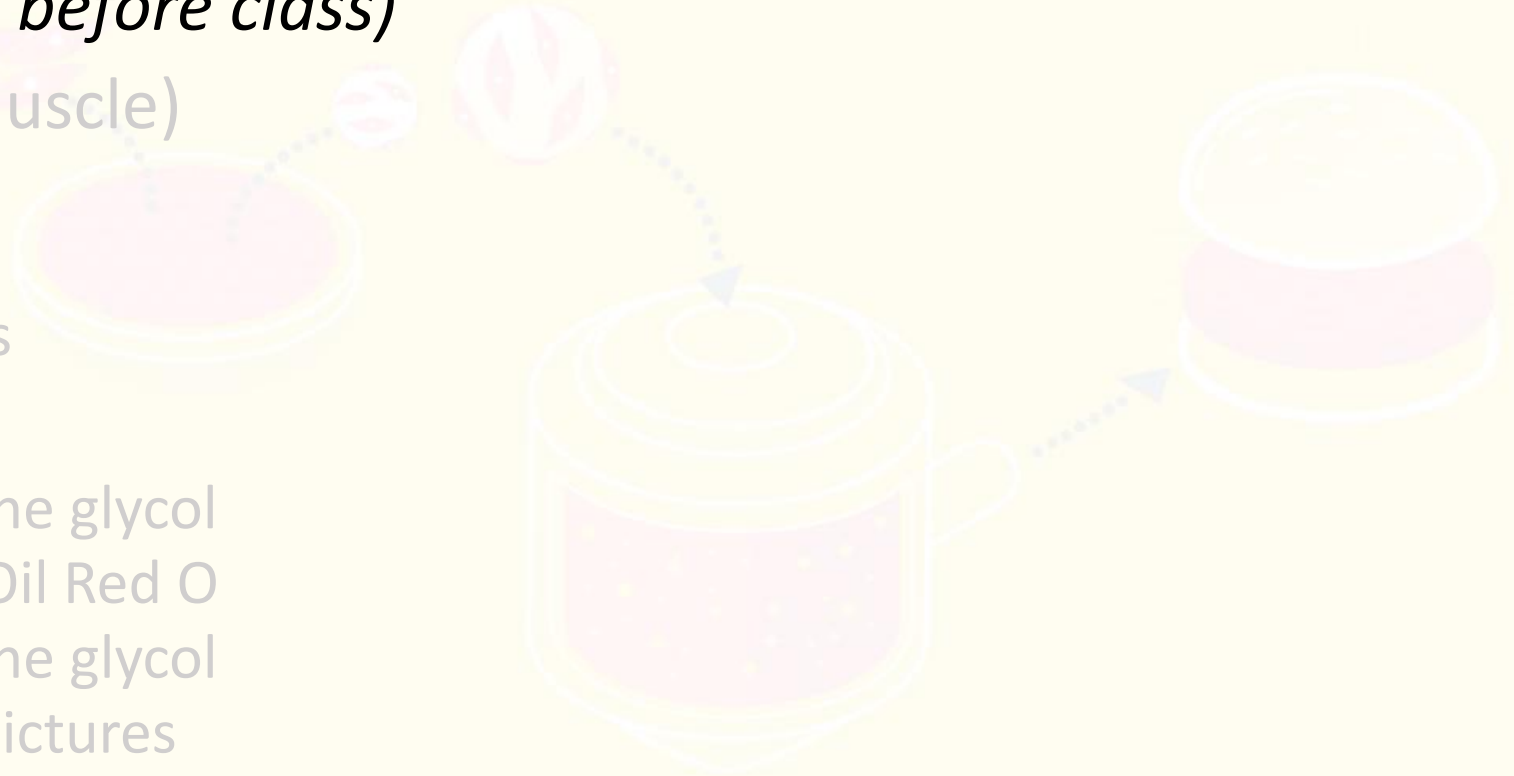
Stains **neutral lipids** via hydrophobic dye

Based on principle that ORO solubility in solvent < ORO solubility in lipids



Today's plan

1. Fix cells (*done before class*)
2. LADD stain (muscle)
 1. Add LADD
 2. Wash
 3. Take pictures
3. Oil Red O (fat)
 1. Add propylene glycol
 2. Incubate in Oil Red O
 3. Add propylene glycol
 4. Rinse, take pictures
 5. Elute Oil Red O & read in plate reader



Today's plan

1. Fix cells (*done before class*)
2. LADD stain (muscle)
 1. Add LADD (stains muscle **dark purple**)
 2. Wash
 3. Take pictures
3. Oil Red O (fat)
 1. Add propylene glycol
 2. Incubate in Oil Red O (stains lipids **red**)
 3. Add propylene glycol
 4. Rinse, take pictures
 5. Elute Oil Red O & read in plate reader

